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Published in:
Biology Letters

DOI:
[10.1098/rsbl.2009.0283](https://doi.org/10.1098/rsbl.2009.0283)

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2009

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Mueller, W., Dijkstra, C., & Groothuis, T. G. G. (2009). Maternal yolk androgens stimulate territorial behaviour in black-headed gull chicks. *Biology Letters*, 5(5), 586-588.
<https://doi.org/10.1098/rsbl.2009.0283>

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Maternal yolk androgens stimulate territorial behaviour in black-headed gull chicks

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Avian eggs contain substantial amounts of maternal androgens. The concentrations of these yolk androgens are affected by the maternal environment, such as the level of social competition, parasite exposure or food conditions. Since yolk androgens have been shown to affect a wide array of offspring traits, they may adjust the chicks to the expected post-hatching environment, but experimental evidence is still scarce. We investigate in colonial breeding black-headed gulls whether high concentrations of yolk androgens, such as those found in environments with high numbers of social interactions, facilitate aggressiveness and territorial behaviour of the chicks. Black-headed gulls are highly suitable for this, as the semi-precocial chicks defend the natal territory and food against intruders. We manipulated yolk androgen concentrations and investigated their role in both within-nest and between-nest aggression. We found that chicks hatching from androgen-treated eggs defended the natal territory more often than their nest mates from control eggs, without increasing sibling aggression. This suggests that variation in yolk androgen concentrations in relation to the social environment of the mother may indeed allow adjustment of the offspring's behaviour to the expected frequency of territorial interactions with conspecifics post-hatching.

Keywords: testosterone; maternal effects; aggression; colonial breeding

1. INTRODUCTION

The effects of yolk androgens on several offspring traits represent examples of a maternal effect, where offspring phenotype is influenced by the maternal transfer of a specific egg component rather than by genetic inheritance (Mousseau & Fox 1998). Most of the offspring traits that are affected by yolk androgens play an important role in sibling competition (reviewed in Groothuis *et al.* 2005; Gil 2008). The systematic within-clutch variation of yolk androgen concentrations with laying order has, therefore, been interpreted as a mechanism for the mother to mediate sibling rivalry (Schwabl 1993; Eising *et al.* 2001). However, differential deposition of yolk hormones may allow females not

only to adjust their offspring differentially to the level of within-brood competition, but also to prepare the entire brood for certain environmental conditions (Schwabl 1997). There is indeed systematic variation of yolk androgens in an among-clutch context, and, in particular, the social environment is an important variable affecting maternal androgen deposition (Groothuis *et al.* 2005; Gil 2008). High levels of social and territorial interactions with conspecifics have been shown to enhance yolk concentrations of maternal androgens (Groothuis *et al.* 2005; Gil 2008).

However, the Darwinian significance of this remains speculative. Social interactions stimulate androgen production (Oliveira 2004), and the enhanced concentrations of yolk hormones may just be an epiphenomenon of elevated androgen production in the mother without benefits for the offspring. However, elevated yolk androgen levels may also yield a direct benefit for the offspring that have to deal with the frequent social interactions with unrelated conspecifics post-hatching, by increasing their competitive behaviour. It has already been shown that yolk androgens play an important role in within-brood (sibling) competition (Groothuis *et al.* 2005; Gil 2008). However, so far the effects of yolk androgens on sibling competition have only been studied in terms of timing of hatching, begging and growth (Groothuis *et al.* 2005; Gil 2008). For between-brood competition, we expected yolk androgens to enhance chick aggressiveness. This is probable, since not only adult but also chick territorial behaviour is modulated by testosterone post-hatching (Ros *et al.* 2002), but data on the effect of yolk androgens on early aggression are as yet entirely lacking.

The colonial breeding black-headed gull (*Larus ridibundus*) represents an excellent model to address this topic. Both adults and chicks are highly territorial, and the precocial chicks stay in the small territories around their nest until fledging. The chicks defend the natal territory and food, which is regurgitated onto the ground by the parents, against intruding conspecific chicks and adults.

2. MATERIAL AND METHODS

(a) General

We conducted this research during May and June 2003, in three sub-colonies of 200–300 breeding pairs within a large black-headed gull colony (6000 breeding pairs, Workumer Waard, The Netherlands). Freshly laid eggs were marked with a non-toxic marker. Modal clutch size is three and androgen concentrations increase within the laying sequence. On the day of clutch completion, we manipulated hormone levels of the first laid egg to resemble those of third laid eggs. Eggs were injected with either 50 µl of vehicle (sterile cold-pressed Sesame oil) or 50 µl of vehicle containing a mixture of 0.12 µg testosterone and 10.0 µg androstenedione (see also Müller *et al.* 2005). Hatching success was 72 per cent for both treatments. The time interval between laying and hatching in days was reduced by androgens in male (control, $n = 23$, 25.13 ± 0.2 ; androgen, $n = 26$, 24.58 ± 0.13 ; Mann Whitney U-test, $p < 0.02$) but not female eggs (control, $n = 28$, 24.54 ± 0.14 ; androgen, $n = 28$, 24.54 ± 0.13 ; Mann Whitney U-test, $p = 0.91$). We created 48 experimental broods, each containing one chick hatching from a sham-treated egg (control-chick) and one chick hatching from an androgen-treated egg (androgen-chick). Experimental broods were created on the day of hatching and the chicks were matched for body mass on hatching (paired sample t -test, $t = 0.03$, $p = 0.97$), and if possible for sex. Body mass was measured every third day. We were able to observe 27 out of the 48 experimental broods (19 out of the 27 were sex-matched). All other broods were not visible from our hides or had already lost one of the chicks prior to the observations starting.

(b) Behavioural observations

All experimental nests were situated within enclosures not containing any other nests (wire mesh, 40–50 cm high) varying in size from 30 to 60 m² containing exclusively experimental nests (14–18 nests). Chicks could freely move within the enclosure. Behavioural observations were conducted from elevated hides 2–3 m away from the enclosures. Behaviour was recorded on a videotape from these hides, without disturbing the activities within the colony. At the end of the field season, the tapes were scored in the laboratory by an observer unaware of the chicks' treatment. Before starting with the observations, the chicks were randomly coloured on either the head, chest or wings to enable individual recognition from a distance. Recordings of the chicks' behaviour started when the chicks were approximately 12 days old, which is the point in time that the parents start to leave the chicks alone for longer time periods. In the absence of their parents, black-headed gull chicks defend a small territory around their nest against intruding neighbours, both chicks and adults, by running towards intruders followed by aggressive pecking (Groothuis 1989). Each bout of such behaviour, performed against intruders as well as siblings, was recorded throughout the observation periods for as long as both chicks were alive. We also recorded when chicks were running over to other territories where parents were regurgitating food for other broods (kleptoparasitism). Observations continued until the chicks were about to fledge (approx. 25 days of age). Each enclosure was observed daily in random order for 1.5 h either in the morning between 08.00 and 11.00 or between 13.00 and 16.00, resulting in about 80 h of observations.

(c) Statistical analysis

Behavioural data were analysed with negative binomial model-fitting using the generalized estimating equations (Zeger & Liang 1986) methodology for clustered data within the GENMOD procedure (SAS/STAT software), applying the log link function and an exchangeable working correlation. All other analyses were performed in SPSS 14.0. For analysing growth, we individually fitted logistic growth curves by least squares regression (SPSS 14.0) for all broods where both chicks survived until fledging ($n = 20$). We used the model: $W = A / (1 + e^{-k(t-t_i)})$, where W is the body mass at a given age, A the asymptotic body mass (g), k (d⁻¹) the logistic growth constant and t_i the point of inflection (d) (Ricklefs 1968).

3. RESULTS

Within their natal territory, androgen-chicks attacked intruders more often than control-chicks (estimate 0.55, s.e. 0.20, $p = 0.007$, figure 1). In one-third of the cases, chicks attacked an intruding adult and in two-thirds of the cases an intruding chick.

As all chicks within an enclosure were experimental chicks, we analysed the proportion of androgen-chicks among the intruding chicks. The entering chick was as likely to be an androgen-chick as a control-chick (estimate 0.41, s.e. 0.55, $p = 0.48$). The frequency of adult aggressive behaviour towards chicks was very low. Adults attacked chicks from other broods in only seven cases (four androgen-chicks and three control-chicks were attacked). Despite the large number of social interactions among siblings, especially when fed by the parents, we observed aggressive behaviour between nest-mates on only one occasion.

Chicks also tried to steal regurgitated food in neighbouring territories. Kleptoparasitic behaviour occurred at a low frequency, and although androgen-chicks performed this almost twice as frequently as control-chicks, this difference was not statistically significant (estimate 0.49, error 0.40, $p = 0.23$, figure 2). Kleptoparasitic behaviour was always followed by an aggressive attack against the stealing chick. Kleptoparasitic behaviour was not included in the analyses of territorial defences/intrusions.

Sex or the interaction of sex and treatment did not have a significant effect on any of the behavioural

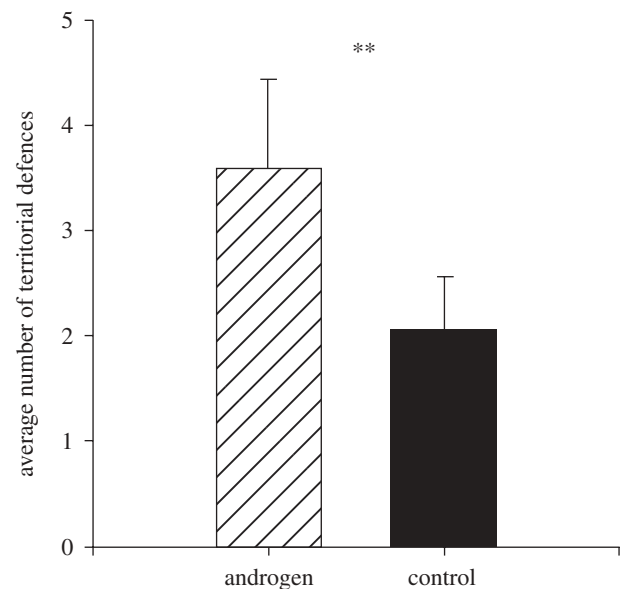


Figure 1. The number of territorial defences was significantly higher for androgen-chicks (cross-hatched bars) than for control-chicks (black bars) (mean \pm s.e.). Asterisks indicate $p = 0.007$.

traits investigated. However, male chicks reached a higher asymptotic body mass than female chicks (linear mixed models, nest as random effect, $F_{1,36} = 6.68$, $p = 0.01$), while there was no significant difference between treatments and no interaction effect of treatment with sex ($p > 0.36$ in both cases).

4. DISCUSSION

Maternal yolk androgen concentrations can be modulated by the environment experienced by the mother (Groothuis *et al.* 2005; Gil 2008). However, experiments testing the adaptive significance of among-clutch variation have rarely been conducted (but see Tschirren *et al.* 2005, 2007; Müller *et al.* 2007). Studies regarding the functional consequences of high clutch levels of yolk androgens such as found in environments with high numbers of social interactions are completely lacking.

We hypothesized that elevated yolk androgen concentrations facilitate the ability of the chick to cope with high levels of between-brood competition by modulating territorial behaviour, and the results of our experiment support this hypothesis. Androgen-chicks were more frequently engaged in the defence of their territory (figure 1). Interestingly, this effect was strongly context dependent, as the level of within-brood aggression was not affected by our treatment. High clutch levels of yolk androgens do not therefore lead to exaggerated sibling rivalry in terms of aggression. Thus, embryonic exposure to elevated yolk androgen concentrations has a positive effect on aggressive behaviour similar to an androgen treatment post-hatching (Groothuis & Ros 2005). Androgen-chicks were not more likely to enter neighbouring territories than control-chicks. Thus, yolk androgens seem to specifically modulate aggression and not indirectly by enhancing general activity.

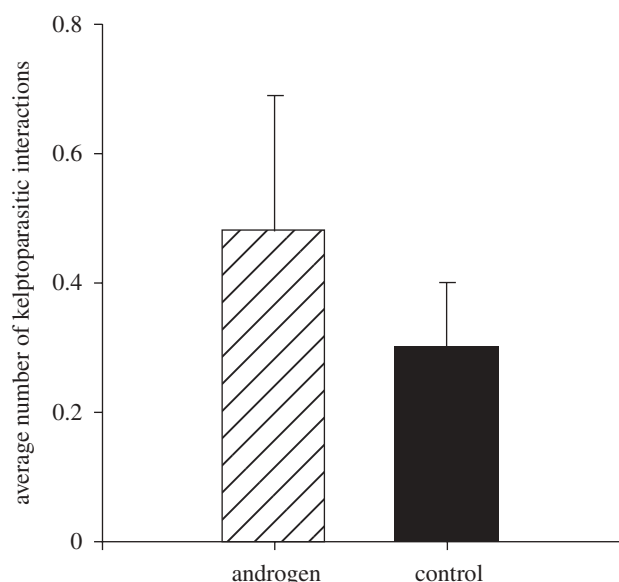


Figure 2. The number of kleptoparasitic behaviours shown by androgen-chicks (cross-hatched bars) and control-chicks (black bars) (mean \pm s.e.).

However, we did not find any significant difference in growth between androgen and control-chicks, as in a previous study (Eising & Groothuis 2003). This may be owing to a reduction in within-brood competition (two instead of three chicks in natural broods in both studies), which probably improved the food conditions, while an effect of yolk androgens on growth was found in the same species using broods of three chicks (Eising *et al.* 2001). Improved food conditions may also be responsible for the low frequency of kleptoparasitism. Furthermore, both chicks may have equally benefited from the enhanced aggressiveness of the androgen-chick. This functional consequence differs evolutionarily from previously reported benefits of yolk androgens at the level of the individual, such as enhanced growth (Groothuis *et al.* 2005). The costs, in contrast to the benefits, of yolk-androgen-modulated nest defence behaviour are likely to be felt individually, e.g. in terms of a higher risk of injury or reduced immunocompetence (Groothuis *et al.* 2005).

In conclusion, this study provides, to our knowledge, the first evidence that high levels of yolk androgens modulate early aggressive behaviour. This suggests that high levels of yolk androgens as a consequence of high levels of social stimulation in the mother, may be adaptive, at least in (semi)-precocial species, by adjusting the offspring to the high level of between-brood competition post-hatching.

This project was approved by the animal experimentation committee of the University of Groningen (DEC 3012).

We thank Dieter Heylen for his statistical support and the referees for their valuable suggestions. W.M. was supported by a PhD studentship (University of Groningen) and by FWO Vlaanderen grant 1.5.033.07.

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